Effects of intravenous and infravesical administration of suramin, terazosin and BMY 7378 on bladder instability in conscious rats with bladder outlet obstruction

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OBJECTIVE

To evaluate the effect of the nonselective purinergic antagonist suramin and the α_1-adrenergic antagonists, terazosin and BMY 7378, given intravenously or infused directly into the bladder during cystometry in conscious rats with bladder outlet obstruction induced by urethral ligation.

MATERIALS AND METHODS

Cystometry was performed in conscious female rats recording bladder volume capacity (BVC), evaluated as the amount of saline infused between two voiding cycles, and micturition volume (MV). Changes in frequency and amplitude of spontaneous non-voiding bladder contractions (NVC) were also recorded. The effects of the intravenous administration of suramin (100 mg/kg), BMY 7378 (1 mg/kg), and terazosin (0.3 mg/kg) on NVC, BVC and MV were evaluated in obstructed rats with bladder infusion of saline. The effects of infravesical infusion of suramin (3–10 μmol/L), terazosin (1 μmol/L) and BMY 7378 (10 μmol/L) were also evaluated and compared with values observed in control rats during saline infusion into the bladder.

RESULTS

Intravenous injection with suramin had no effects on NVC, BVC and MV, but suramin infused into the bladder induced a consistent reduction in the amplitude of NVC (significantly different from matched control animals) with a tendency to reduce their frequency. BVC and MV were slightly but significantly decreased by infravesical infusion of suramin. In contrast, BMY 7378 and terazosin, given intravenously, were extremely potent at inhibiting the frequency and amplitude of the NVC, but were inactive on NVC when infused into bladder.

CONCLUSIONS

These findings confirm a role for α_1-adrenergic receptors in bladder instability caused by bladder outlet obstruction. In addition, a purinergic neurotransmitter, presumably ATP, is shown to be involved.

KEYWORDS

purinergic antagonists, α_1-antagonists, cystometry, micturition reflex.

INTRODUCTION

In rats, bladder hypertrophy secondary to BOO induces bladder instability, characterized by the presence of non-voiding contractions (NVC) during filling, mirroring the clinical condition in humans with BOO. These NVC seemed to be related to the irritative symptoms frequently observed in patients with BOO secondary to BPH. Although several studies have attempted to characterize the functional changes in the bladder caused by BOO, bladder instability after obstruction has not been well characterized so far in conscious rats. In particular, few studies have reported the effect of pharmacological treatments on NVC [1–6].

The involvement of ATP in nonadrenergic, noncholinergic (efferent) contraction of the urinary bladder is well documented [7,8]. Recently, it was also shown that ATP is released from the urothelium of isolated urinary bladder following increased intraluminal pressure [9]. Birder *et al.* [10] showed that stretch-evoked ATP release was diminished in bladders excised from 'knockout' mice lacking the vanilloid receptor *TRPV1*, indicating that this capsaicin-gated ion channel is essential for normal mechanically evoked purinergic signalling by the urothelium. Thus, non-neuronally released ATP could also be involved in the initiation and/or maintenance of NVC. Calvert *et al.* [11] showed that the purinergic component of nerve-mediated detrusor contraction is increased in a model of BOO in rabbits. Furthermore, Bayliss *et al.* [12] reported atropine-resistant, nerve-mediated contractions in strips of bladder from patients undergoing TURP. They found that this component was abolished after desensitizing the strips with α,β-methylene ATP, suggesting that it was mediated by neurally released ATP acting on ligand-gated purinergic receptors. Accordingly, Igawa *et al.* [2] showed that intra-arterial α,β-methylene ATP reduced the frequency and amplitude of NVC in rats with BOO.

During the last few years, much attention has been focused on the role of the sympathetic nervous system and α_1-adrenergic receptors in the smooth muscle contraction component of BOO. Three α_1-adrenergic receptor subtypes (α_{1a}-, α_{1b}-, and α_{1d}-) have been cloned and pharmacologically characterized [13]. Recently [14] it was shown that while overall total α_1-adrenergic receptor expression remained stable, there were dramatic alterations in α_1-adrenergic receptor subtype expression with BOO. Whereas the α_{1a}-adrenergic receptor subtype predominated in the bladder of unobstructed rats, after 6 weeks of obstruction the α_{1d}-adrenergic

subtype predominated, being newly expressed at the protein level.

In view of the purinergic/adrenergic involvement in BOO, the aim of the present work was to evaluate the effect of the nonselective purinergic receptor antagonist, suramin, on NVC in conscious, obstructed rats, compared with the effects of the subtype unselective α_1-adrenergic antagonist terazosin, and BMY 7378, a compound with selective antagonistic activity at the α_{1d}-adrenergic receptor subtype [15]. A preliminary account of some of these results was presented in abstract form [16].

MATERIALS AND METHODS

Female Sprague-Dawley rats (Crl: CD® (SD) BR) weighing 225–275 g (Charles River, Italia) were used; they were housed with free access to food and water and maintained on forced 12 h light-dark cycle at 22–24°C, except during experiments. The animals were handled according to internationally accepted principles for care of laboratory animals (EC Council Directive 86/609, OJ no L358, 18/12/86). Cystometrography was undertaken using the procedures previously reported [1,6], with some modifications.

Rats were anaesthetized by intraperitoneal administration with 2 mL/kg of equithensin solution (pentobarbital 1.215 g, chloral hydrate 5.312 g, magnesium sulphate 2.657 g, ethanol 12.5 mL, propylene glycol 49.5 mL, distilled water to 125 mL final volume) and then the bladder and proximal urethra exposed via a lower abdominal midline incision. A silk ligature was placed around the urethra and tied in the presence of an intraluminal indwelling polyethylene cannula with an outside diameter of 1.22 mm. After removing the polyethylene cannula, the abdominal wall was sutured and then antibiotic medication (penicillin G 200 000 IU/kg and streptomycin 300 000 IU/kg intramuscular) administered. Three weeks after obstruction the animals were prepared for cystometry.

For cystometry, the animals, anaesthetized as reported above, were placed supine and a midline incision made in the shaved and cleaned abdominal wall. The urinary bladder was exposed and gently freed from adhering tissues, emptied and then cannulated, via an incision at the dome, with a polyethylene cannula (0.58 mm internal, 0.96 mm outside diameter), which was permanently sutured with silk thread. For intravenous bolus injection a similar polyethylene tube filled with physiological saline and containing sodium heparin (40 IU/mL) was inserted into the jugular vein. The cannulae were exteriorized through a subcutaneous tunnel in the retroscapular area, where they were connected with a plastic adapter, to avoid the risk of removal by the animal.

Two days after implanting the bladder catheter the animals, fasted overnight, were placed in plastic Bollman's cages which allow the rats some lateral and back and forth movement. After a stabilization period of 20 min, the free tip of the bladder cannula was connected to a pressure transducer (P23 XL, Viggo-Spectramed, Exnard, CA, USA) and to a peristaltic pump (Minipuls 2, Gilson-Italia, Milan, Italy) for continuous infusion of warm saline (37°C) into the urinary bladder; the filling rate chosen was 10 mL/h. During infusion of saline into the bladder, intravesical pressure and urine volume voided were recorded continuously using a computer interface and appropriate software. Test compounds were administered after 1 h of saline infusion, to acquire basal values before treatment.

From the cystometrograms of the obstructed rats (e.g. Fig. 1), the number and mean amplitude of the spontaneous bladder contractions present during bladder filling with no urine emission and termed NVC were evaluated for the 2 min before voiding. In addition, bladder volume capacity (BVC) and micturition volume (MV) were evaluated. The BVC was defined as the volume of saline infused into the bladder from the start of the infusion to the time when detrusor contraction was followed by voiding. The MV was the volume of expelled urine during each single void, recorded by a force-displacement transducer connected to the recording polygraph, and measuring the urine collected in a small reservoir placed under the cage (Fig. 1). As the ligature around the urethra was not removed before cystometry, peak micturition pressure was not considered in these experiments.

The variables (BVC and MV) are reported as the mean values obtained from individual cystometrograms recorded for 1 h before and 1 h after starting the intravesical infusion or intravenously administered test compounds. Suramin (Sigma-Aldric, Milan, Italy), terazosin and BMY 7378 (both Recordati SpA, Milan, Italy) were given intravenously at doses of 100, 0.3 and 1 mg/kg, respectively. The dose of suramin was chosen on the basis of previously published results [24] showing that suramin was active at this dose in obstructed rats. Higher doses of suramin were not administered because the compound is toxic. The doses of terazosin and BMY 7378 used for intravenous administration were chosen from

*FIG. 1. Typical cystometrographic traces obtained in a conscious rat with BOO, showing the cystometric variables analysed. The upper trace represents intravesical pressure and the lower represents MV. Several NVC are apparent before micturition; *indicates adjustment to the baseline position.*

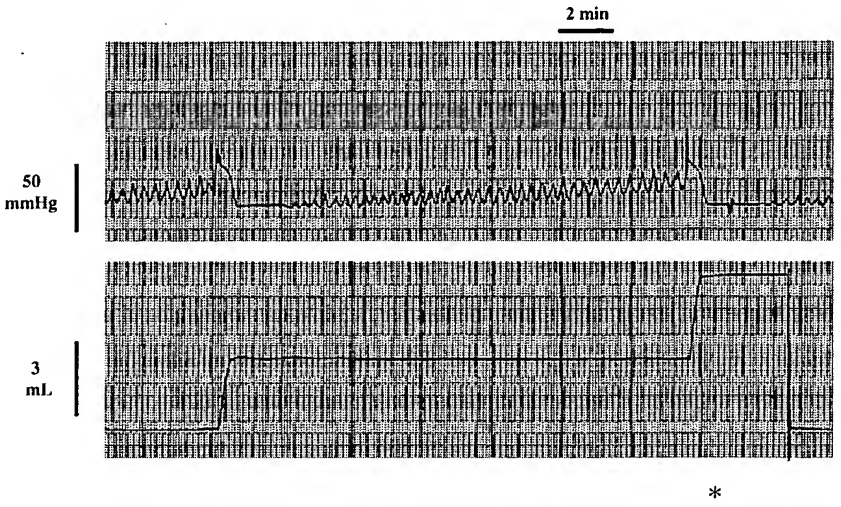


TABLE 1 Effects of intravenous and intravesical administration of suramin, BMY 7378 and terazosin on cystometrographic variables, as the mean (SEM), in obstructed rats.

Group [N]	NVC Frequency, n/2 min	NVC Amplitude, mmHg	BVC, mL	MV, mL
Intravenous				
Controls [7]				
Before	5.3 (0.9)	5.2 (0.9)	1.27 (0.16)	1.36 (0.24)
After	4.9 (0.9)	4.9 (0.8)	1.16 (0.25)	1.31 (0.28)
Suramin 100 mg/kg [4]				
Before	4.4 (0.3)	5.8 (0.8)	2.27 (0.23)	1.88 (0.32)
After	4.8 (0.2)	5.9 (0.6)	2.26 (0.39)	2.30 (0.47)
BMY 7378 1 mg/kg [8]				
Before	6.6 (0.7)	10.0 (1.0)	1.79 (0.24)	1.69 (0.25)
After	3.5 (0.6)†	6.3 (0.7)†	1.51 (0.24)	1.69 (0.27)
Terazosin 0.3 mg/kg [7]				
Before	5.4 (0.5)	10.3 (1.9)	2.44 (0.39)	2.22 (0.22)
After	2.5 (0.4)†	5.8 (0.7)*	2.47 (0.68)	1.79 (0.34)
Intravesical				
Controls [10]				
Before	5.1 (0.3)	6.7 (1.0)	1.65 (0.26)	1.71 (0.37)
After	4.8 (0.4)	6.3 (0.8)	1.64 (0.26)	1.52 (0.22)
Suramin 10 μmol/L [9]				
Before	5.3 (0.3)	9.9 (1.6)	1.72 (0.25)	1.71 (0.30)
After	4.5 (0.6)	7.0 (0.8)*	1.46 (0.21)*	1.47 (0.25)†
BMY 7378 10 μmol/L [8]				
Before	5.5 (0.9)	9.6 (2.1)	1.77 (0.23)	1.56 (0.18)
After	5.0 (0.9)	8.8 (1.9)	1.82 (0.18)	1.91 (0.20)*
Terazosin 1 μmol/L [6]				
Before	5.3 (0.3)	9.6 (1.1)	2.36 (0.46)	2.18 (0.47)
After	5.5 (0.7)	9.4 (1.2)	1.93 (0.33)	1.88 (0.26)

$P < 0.05$; †$P < 0.01$, before vs after administration.

our pilot experiments and other published data [4]. For bladder infusion, 3 and 10 μmol/L of suramin were used, chosen on the basis of positive results previously obtained in unobstructed rats [17]. The concentration of terazosin (1 μmol/L) and BMY 7378 (10 μmol/L) were chosen considering the results obtained after the intravenous administration of these compounds in comparison with those of suramin.

For intravenous administration, suramin was dissolved in saline and infused over 10 min, whereas terazosin and BMY 7378 were dissolved in distilled water and administered as a bolus injection. For bladder infusion, the compounds were dissolved in saline and infused into the bladder for 1 h.

The differences between the cystometrographic measures recorded before and after intravesical infusion or intravenous administration of compounds were analysed statistically by Student's *t*-test for paired values. The difference between treatments was evaluated as the change (Δ, after – before) values of each variable using ANOVA and Dunnett's test.

RESULTS

In the experimental conditions the weight of the bladder from the rats with partial urethral ligation was ≈500 mg, whereas the corresponding value in normal rats was ≈140 mg, confirming the presence of hypertrophy induced by obstruction, as reported previously [18].

Repeated cystometry with saline in control obstructed rats gave reproducible results for the frequency and amplitude of spontaneous contractile activity (NVC). BVC and MV in these animals were also not significantly changed during the observation period (Table 1).

After intravenous administration, suramin (100 mg/kg) had no activity on cystometrographic variables. BMY 7378 (1 mg/kg) and terazosin (0.3 mg/kg) markedly and significantly decreased both the frequency and the amplitude of NVC (Table 1). There were no significant changes in BVC and MV.

Infusing 3 μmol/L of suramin into the bladder did not change the cystometrographic values (data not shown). In contrast, suramin 10 μmol/L induced a statistically significant decrease of the amplitude of NVC, and a trend toward a decrease in frequency of contractions compared with basal values (within rats). BVC and MV were slightly but significantly decreased by suramin.

There were no significant changes in NVC in bladders infused with BMY 7378 (10 μmol/L) or terazosin (1 μmol/L). BMY 7378 slightly increased MV, whereas terazosin did not significantly change BVC and MV (Table 1). The concentration of BMY 7378 and terazosin infused into the bladder was higher than the concentration of infused suramin. The ratio between the intravenous dose of suramin (100 mg/kg, inactive) and its active concentration after infravesical infusion (10 μmol/L) was 10. Maintaining the same ratio for BMY 7378 and terazosin (active at 1 and 0.3 mg/kg intravenously, respectively) the concentrations that should have been infused would be 0.1 and 0.03 μmol/L, respectively, instead of 10 and 1 μmol/L.

The effects of infravesical and intravenous administration of the compounds on the frequency and amplitude of NVC, expressed as the percentage change from basal values are shown in Fig. 2. Statistical analysis confirmed that infravesically administered suramin induced a significant decrease in the amplitude of NVC compared with the control, whereas BMY 7378 and terazosin were markedly active after intravenous administration but had no activity when delivered infravesically. There were no significant differences in Δ values between control and treated groups in the other cystometrographic variables (data not shown).

DISCUSSION

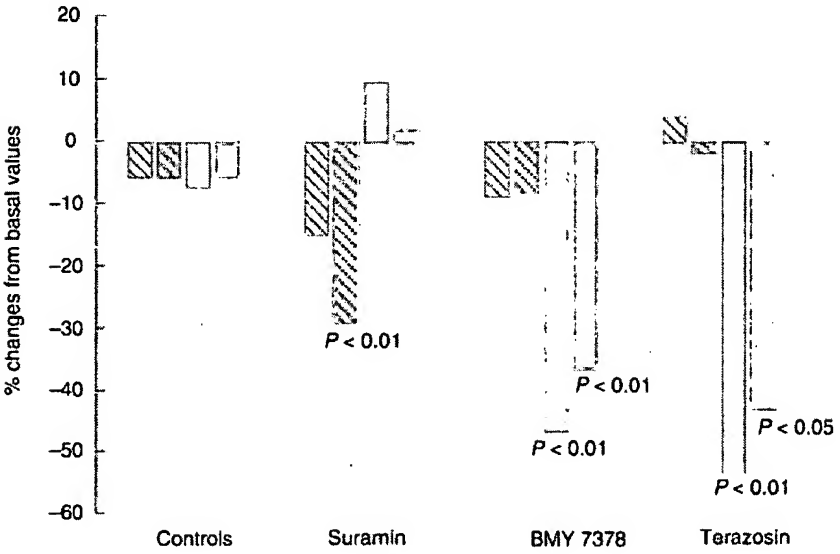
Bladder overactivity, a major cause of urinary incontinence, is often associated with an unstable detrusor that is characterized by involuntary contractions which generally do not lead to emptying of the bladder. BOO caused by BPH in men causes bladder instability that persists in about a quarter of patients, even after the surgical relief of the BOO [19]. There is still no consensus about the cause of detrusor instability after BOO. Sensitization of peripheral afferent nerve terminals in the bladder, and damage to central inhibitory pathways unmasking primitive voiding reflexes that can trigger bladder overactivity, may be involved [20]. Furthermore, Brading [21] proposed that all cases of detrusor instability have a common feature, i.e. a change in the properties of the smooth muscle of the detrusor predisposing it to unstable contractions. If this hypothesis is correct, the primary functional change in the detrusor in response to BOO is an increased excitability of smooth muscle.

Animal models of infravesical obstruction have been developed in several species, and previous investigations of bladder function during BOO in rats have shown that a condition similar to that seen in patients with BPH develops in these animals, characterized by the presence of NVC in detrusor muscle during the filling phase of the bladder [22–24]. Several reports show these contractions can be blocked by calcium antagonists, potassium channel openers and α_1-adrenoceptor antagonists [1–5].

The present results confirm that the subtype unselective α_1-adrenergic antagonist terazosin and the α_{1d}-adrenergic receptor antagonist BMY 7378 were markedly active in reducing NVC after intravenous administration, in agreement with previously reported data [4]. On the contrary, the two α_1-adrenergic receptor antagonists had practically no effect on NVC after infravesical infusion, a finding that to our knowledge has not been published before.

Previous studies have indicated that intrathecal doxazosin (a subtype unselective α_1-adrenergic receptor antagonist) had no effect on the frequency or amplitude of the unstable contractions in rats with BOO [25]. Similarly, intra-cerebroventricular administration of prazosin and terazosin had no effect on uninhibited NVC [26]. However, in the same animals these compounds produced an increase in BVC and, sometimes, dribbling incontinence, suggesting an action at supraspinal and spinal centres containing α_1-adrenergic receptors contributing to voiding control.

FIG. 2. Effect of various infravesical (hatched bars) and intravenous (open bars) treatments on the frequency (green) and amplitude (red) of NVC in obstructed rats. Data represent the percentage changes after treatment from the basal values. The statistical significance of Δ was evaluated as reported in the methods, vs the corresponding Δ of the control group.



In the present experiments, intravenous terazosin and BMY 7378 blocked NVC at a dose that did not modify BVC and MV, suggesting that unstable contractions are more sensitive to α_1-blockade than the other voiding variables. However, the lack of activity of α_1-adrenergic receptor antagonists directly infused into the bladder and their potency when injected intravenously suggests that α_1-adrenergic receptors are not located at the level of afferent nerve endings within the urothelium.

It was recently suggested [14] that α_{1d}-adrenergic receptor subtype could be relevant for onset of bladder instability. Terazosin (an α_1-adrenergic receptor antagonist showing the same affinity for all the α_1-adrenergic receptor subtypes) and BMY 7378 (a selective α_{1d}-adrenergic receptor antagonist) have the same affinity for the α_{1d}-adrenergic receptor subtype [15]. Nevertheless, terazosin (0.3 mg/kg intravenous) was as potent as BMY 7378 (1 mg/kg) as an inhibitor of NVC. Although a full dose-response curve should be evaluated to compare the two compounds, these findings seem to suggest that a block of the α_{1d}-adrenergic receptor subtype alone is not sufficient to efficiently inhibit bladder instability.

The infravesical infusion of the nonselective P2 purinergic antagonist suramin (10 μmol/L) in rats with bladder overactivity caused by BOO induced a significant decrease in the amplitude of NVC. The change was statistically significant both from basal values (before treatment) and compared with changes recorded in a matched control group with bladders infused with saline.

Although the involvement of ATP in nonadrenergic, noncholinergic (efferent) contraction of the urinary bladder has been reported [8], only in recent reports has it been shown that ATP is also released from isolated urinary bladder urothelium after increased intraluminal pressure [9]. Igawa *et al.* [2] investigated the purinergic components of detrusor contraction in conscious rats with BOO by continuous cystometry, showing that the amplitude of NVC decreased significantly and their frequency tended to decrease after the intra-arterial administration of α,β-mATP. Furthermore, it has been reported that

infravesical ATP stimulates the micturition reflex in awake, freely moving rats [27] and that during cystometry the number of impulses generated in the afferent neurones was halved by treatment with suramin [28]. As the ATP receptor P2x3 is critical for afferent pathways controlling urinary bladder volume reflexes [29], the activity of suramin in the present experimental conditions could be related to its antagonistic activity at these receptors. Alternatively, it has been reported that hyperpolarization of the detrusor muscle cells by opening K^+ channels may reduce bladder excitability, and it is well established that drugs opening K_{ATP} channels inhibit NVC in obstructed rats [2,3]. Extracellular ATP inhibited the K_{ATP} channel current and ATP-induced channel inhibition was hardly detected in the presence of suramin [30]. Therefore it is possible that the inhibition of NVC induced by suramin infusion into the bladder is exerted by counteracting the ATP-induced inhibition of K_{ATP} channels at urothelial level.

In the present experimental conditions the intravenous administration of suramin (100 mg/kg) did not change the frequency and amplitude of NVC. As it has been previously reported that the same dose of suramin in conscious control and obstructed rats significantly decreased peak micturition pressure [24], it is plausible that only purinergic receptors on epithelial cells, acting on the afferent nerves penetrating the basal lamina, are involved in the activity of infravesical suramin on NVC.

In conclusion, the present findings suggest that, in rats with BOO, the NVC indicative of bladder instability are sensitive to purinergic antagonists acting at the urothelial level, and to α_1-adrenergic receptor antagonists acting at a site distant from the urothelial cells.

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Abbreviations: **NVC**, non-voiding contractions; **BVC**, bladder volume capacity; **MV**, micturition volume.